The European dimension for the mouse genome mutagenesis program

Published in final edited form as:
Nat Genet. 2004 September ; 36(9): 925–927. doi:10.1038/ng0904-925.

The European dimension for the mouse genome mutagenesis program

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URLs. The German Genetrap Consortium is available at http://www.genetrap.de/. The Sanger Institute Gene Trap Resource is available at http://www.sanger.ac.uk/genetrap/. The Harwell Mouse Mutagenesis Programme is available at http://www.mgu.har.mrc.ac.uk/. The Institut Clinique de la Souris is available at http://www-mci.u-strasbg.fr/.

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Abstract

The European Mouse Mutagenesis Consortium is the European initiative contributing to the international effort on functional annotation of the mouse genome. Its objectives are to establish and integrate mutagenesis platforms, gene expression resources, phenotyping units, storage and distribution centers and bioinformatics resources. The combined efforts will accelerate our understanding of gene function and of human health and disease.

The sequencing of mammalian genomes has shown that their gene catalogs are unexpectedly small, with ~28,000 genes. Owing to the development of mouse embryonic stem (ES) cells1,2 and a variety of mutagenic technologies3-12, it is now possible to plan a systematic assault on the mouse genome to document function. The most straightforward way to start this program is to create and characterize null alleles in the germ line. But null mutants in the germ line identify only the earliest function in development, and later functions are often occluded. Furthermore, many genes are expressed with temporal and spatial (tissue) specificities, and many proteins are expressed as alternative or post-translationally modified forms. Therefore, a straightforward analysis of 28,000 null-allele mutants will be far from functionally exhaustive. Strategies for conditional mutagenesis in the mouse permit the discovery and dissection of gene function throughout the life cycle and in a chosen cell type. Because health and disease are often related to aging, spatially and temporally controlled conditional mutagenesis is also crucial for the medical and social relevance of mutational studies in the mouse. Therefore, a systematic program of functional documentation in the mouse will ideally combine the advantages of null- and conditional-allele approaches while recognizing the disadvantages of each.

A comprehensive assembly of mammalian gene functional data is a far-reaching project that will require an extraordinary range of expertise and tools. It will depend on the maximal integration of basic biological and clinical data alongside the development and application of efficient and standardized methodologies.

Already, discussions in the international mouse research community have considered the new challenges and underlined the importance of large-scale mouse mutagenesis and its potential benefit to biomedical science13,14. The scale of a comprehensive undertaking to add functional understanding to the mammalian gene catalog clearly requires the development of cooperative structures akin to present-day research consortia in physics and astronomy. In Europe, the requirement for integrated programs to tackle elements of the greater task has been met so far by the establishment of various national and European Community–funded programs including EUMORPHIA, an integrated network of centers for standardized phenotyping; EURExpress15,16, a program to document thousands of expression patterns at mid-gestation allied to EMAGE, the mouse atlas project; the German and British Gene Trap Consortia, parts of the International Gene Trap Consortium, which has established banks of ES cells mutated with sequence-verified gene traps17; ENU mutagenesis centers in Germany and the UK, undertaking broad-based phenotype-driven mutagenesis programs; European conditional mouse mutagenesis centers, undertaking spatially and temporally controlled, site-specific somatic mutagenesis; the European mouse mutant archive (EMMA); and the European Bioinformatics Institute (EBI), for database generation and dissemination of results. Since December 2002, several European Community–sponsored pan-European discussions have focused on further development and

coordination of mouse functional genomics in Europe. From these discussions has emerged Priorities for Research in Mouse Genetics in Europe (PRIME), a forum whose remit has been to weigh the new challenges, consider the opportunities and economies of greater coordination and build on existing accomplishments and resources. In two meetings (15 July 2003 and 9 January 2004), PRIME agreed on a primary focus for European efforts in mouse genome mutagenesis.

A European mouse mutagenesis program

We present an initial working plan for a pan-European effort in mouse mutagenesis that builds on existing resources in mutagenesis, phenotyping, expression studies and informatics to make a first step towards a comprehensive annotation of gene function in the mouse genome. There are several important early priorities of this plan: (i) continued work to establish a complete catalog of null reporter alleles in ES cells through the International Gene Trap Consortium; (ii) further development of centers that archive and distribute mutant resources (ES cell lines, sperm, oocytes; these centers will also generate mouse lines from the mutant resources and document initial aspects of phenotype); and (iii) integration of European mouse functional genomics and expression pattern data sets, logically through EBI, with other international initiatives to create a unified global database.

The integration of European mouse research programs with international initiatives to create a coordinated global research program will maximize the collective effort and public access and minimize redundancy. The early establishment of an efficient international infrastructure and standardized, user-friendly practices will deliver downstream benefits for the more demanding challenges to come. Furthermore, existing mutant resources from the German and British gene trap programs and the European Community–sponsored EUMORPHIA, as well as expression and functional data from EURExpress and other programs, should be built into a unified, publicly accessible, international effort as soon as possible.

EUCOMM

Conditional mutagenesis in Europe is strong, in large part due to the local development of key technologies, including applications of site-specific recombination3,8,12 and ligand-inducible switches18-21. Because conditional mutagenesis is required for the accurate functional analysis of most genes, it is rational to build the European contribution to international efforts on existing European strengths.

The goals of the European Conditional Mouse Mutagenesis Program (EUCOMM) are as follows: (i) develop generic conditional strategies, using conditional gene traps and multipurpose targeting cassettes, for the rapid creation of conditional alleles in ES cells; (ii) establish dedicated gene targeting and conditional gene trap units; (iii) use gene targeting with multipurpose conditional alleles to help complete the null reporter allele set; (iv) comprehensively expand, archive and distribute the collection of transgenic mouse lines expressing Cre recombinase; (v) focus coordinated efforts of conditional mutagenesis and phenotyping on particular subsets of genes (including, for example, genes known to be involved in human disease or new mammalian transcribed sequences); (vi) continue to explore and incorporate technology advances in functional genomics (*e.g.*, the use of point mutagenesis, RNA interference and other emergent technologies that operate at the RNA level) to complement null and conditional mutagenesis and enrich the depth of the mutant resources.

Conditional analyses require a greater investment than does null mutagenesis in the germ line. Therefore, it makes sense at this stage to prioritize genes for conditional mutagenesis

and phenotyping. For example, one possible focus of EUCOMM might be the OMIM list of genes known to be associated with human diseases, because they are well-suited to analysis using the power of conditional mutagenesis and are of medical relevance. The development of 'recombineering' technology and the availability of bioinformatics resources as an outcome of the genome-sequencing project have greatly reduced the time and costs involved in the establishment of targeted conditional alleles22-25. Nevertheless, to generate a large number of conditional alleles efficiently, gene-targeting units need to be established. These units will complement existing European strengths in gene traps, expression analysis, ENU mutagenesis and phenotyping26,27 and will add capacity for the economical production of precise mutations, such as point mutations or isoform-specific knockouts. They will also facilitate the establishment of the complete null-allele set by targeting those genes that prove recalcitrant to mutagenesis by gene trapping. The collection of transgenic mouse lines expressing Cre recombinase needs to be expanded, most efficiently by exploiting characterized expression patterns and taking advantage of cassette exchange technology, and housed in central distribution centers such as EMMA. In addition, EUCOMM aims to maintain a strong emphasis on further technology development so that the program can rapidly incorporate improvements that facilitate achievement of the overall goals. Such technology developments include alternative approaches to mutagenesis, such as ENU gene-driven screens or conditional RNA knock-down strategies.

Planting FLAGs in the mouse genome

A main goal of the mouse mutagenesis program is to promote hypothesis-driven research by as broad a range of scientists as possible, including not only experienced mouse researchers but also those who do not have the facilities or expertise to generate mice from ES cells. Therefore, the archiving and distribution centers will also generate mice. Furthermore, by harnessing mouse production to initial phenotyping to add FLAGs (first-line functional annotations of the genome) to the mutant collection, we will enhance hypothesis-driven use of the mutagenesis resources.

We envisage that the existing European distribution centers for mouse strains will expand to archive and distribute the mutant resources. New centers may also emerge, and all will need to be coordinated, logically under the auspices of EMMA. The centers will not only archive and distribute ES cells, but also use their expertise to generate mice and freeze gametes for distribution. Priorities for the generation of mice from ES cells will be determined by demand from committed scientists who will pay a cost-covering charge and by the gene subsets selected by EUCOMM for systematic conditional mutagenesis. Many of these centers are also involved with the development and application of high-throughput phenotyping platforms, in particular the development of new first-line screens such as the European Comprehensive First-Line Phenotyping protocol. We therefore envisage that these centers will engage in both resource and phenotyping activities to document a first-line annotation of the mouse genome. For mouse production, the centers will generate heterozygous mutant mouse lines and then breed them, to secure embryos and sperm for archiving and further dissemination, to determine developmental lethality in the homozygote and to apply, where appropriate, first-line phenotyping screens. In addition, the centers may interact with dedicated units that document heterozygous reporter expression patterns in a generic manner. Providing first-line phenotyping information on any scale will place a considerable demand on the existing phenotyping platforms. But the synergies between archiving, distribution and phenotyping and the opportunity, as each mouse is generated, to develop FLAGs, will add substantial value to each mutant and stimulate individual researchers to acquire mice of interest and embrace further analysis. Even the archiving and dissemination of these new ES cell resources will require a considerable investment in and expansion of existing facilities in Europe.

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The principles governing access to the mouse mutant resource are those that have been applied to the human genome sequencing program. The science is best served by unrestricted access to all the resources generated and rapid deposition of information into the public domain.

The global context

The European priorities elaborated here complement the initiative launched at the Banbury meeting on the Knockout Mouse Project (from 30 September to 1 October 2003; ref. 28). In this paper, The Comprehensive Knockout Mouse Project Consortium outlines a hierarchy of practical steps, dividing goals into short- and long-term objectives. There is substantial concordance between the Knockout Mouse Project and EUCOMM with regard to the short-term objectives, particularly those relating to the establishment of a centralized bioinformatics platform for data deposition and a global expression atlas, as well as extending capacities for central storage and distribution centers for mice and ES cells. In addition, European efforts in gene trap mutagenesis, as part of the International Gene Trap Consortium, will contribute substantially to the planned bank of 28,000 null reporter alleles in ES cells. Due to existing European strengths in conditional mutagenesis and phenotyping platforms, the European proposal differs from the Knockout Mouse Project proposal with respect to overall strategy and priority emphasis. We regard this diversity as a strengthening factor for the forthcoming international collaboration. The immediate task at hand is to begin the process of coordination and integration, developing detailed structural plans and harnessing the wider expertise of the community, so that this milestone can be achieved with speed, quality and economy.

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